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# Relationship of colonization and sporulation by VA mycorrhizal fungi to plant nutrient and carbohydrate contents*

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SUMMARY

Vesicular–arbuscular (VA) mycorrhizal fungi typically produce spores either within roots of a host plant or in the soil. Experiments were conducted to examine the effects of plant nutrition, as measured by tissue N, P, K, and soluble sugar concentrations, upon colonization of roots of *Paspalum notatum* Flügge and sporulation within roots by *Glomus intraradix* Schenck & Smith and in the soil by *Acaulospora longula* Spain & Schenck and *Gigaspora margarita* Becker & Hall. Plants receiving a balanced nutrient solution without P consistently had the greatest percentage root length colonized by VA mycorrhizal fungi. *Glomus intraradix* produced more spores per root weight under conditions of nonmycotrophy, when plants received either water only or $KH_2PO_4$ only in the nutrient–poor soil used in these experiments. *Gigaspora margarita* and *A. longula* produced more spores in the soil when plants received the balanced nutrient solution without P. Similarly contrasting responses were seen when sporulation inside and outside the roots was correlated to plant nutrient and soluble sugar concentrations. The P–tolerant, intraradical–sporulating, VA mycorrhizal fungus *G. intraradix* sporulated heavily when N:P ratios of host tissue were imbalanced toward P. *Acaulospora longula* and *G. margarita* produced more spores in the soil when plant tissue N:P ratios were imbalanced toward N.

Key words: Vesicular–arbuscular mycorrhizas, *Glomus intraradix*, *Acaulospora longula*, *Gigaspora margarita*, nutrients.

## INTRODUCTION

Colonization of plant roots by vesicular–arbuscular (VA) mycorrhizal fungi has been studied in relation to P nutrition and carbohydrate content of roots. The correlation between P nutrition and root exudation upon colonization has been well documented (Graham, Leonard, & Menge, 1981; Schwab, Menge & Leonard, 1983*a*). The relationship between colonization and soluble sugar content of roots is not clear, however. Percentage root length colonized by VA mycorrhizal fungi was correlated with soluble sugar content of roots (Jasper, Robson & Abbott, 1979; Johnson *et al.*, 1982) and the effect of P upon colonization may be due to decreased sugar content in roots (Same, Robson & Abbott, 1983; Thomson, Robson & Abbott, 1986). However, both lower and higher sugar concentrations have been measured for roots of *Sorghum vulgare* Pers. receiving P relative to roots not receiving P (Schwab, Menge & Leonard, 1983*b*, Ratnayake, Leonard & Menge, 1978, respectively). Further, Ocampo & Azcon (1985) studied wheat cultivars with differing susceptibilities to VA mycorrhizal fungus colonization and found no correlation between colonization and sugar content of roots.

Colonization of roots by VA mycorrhizal fungi is also related to general plant nutrition and nutrient availability. The availability of nutrients can be manipulated to provide optimal conditions for symbiosis with P flow from fungus to plant and C flow from plant to fungus (Bethlenfalvay *et al.*, 1982). Nitrogen or nutrient solutions without P have enhanced colonization of roots (Hepper, 1983; Verkade & Hamilton, 1983; Thompson, 1987).

Sporulation of VA mycorrhizal fungi is not necessarily related to the degree of colonization of the host root system (Hetrick & Bloom, 1986; Coltman, Waterer & Huang, 1988). Nor is it consistently related to P fertilization since applications of P to pot cultures of VA mycorrhizal fungi can decrease (Menge *et al.*, 1978; Kiernan, Hendrix

**Table 1.** *Concentrations of macronutrients in nutrient solutions applied to* Paspalum notatum *seedlings colonized by VA mycorrhizal fungi*

| Solution number | N:P index* | Concentration (mg kg$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | N | P | K | Ca | S | Mg |
| 1 | 0:0 | 0 | 0 | 0 | 0 | 3·7 | 0 |
| 2 | 2:0 | 196 | 0 | 234 | 160 | 64·0 | 48 |
| 3 | 1:2 | 105 | 31 | 137 | 100 | 32·0 | 24 |
| 4 | 2:2 | 196 | 31 | 273 | 160 | 64·0 | 48 |
| 5 | 0:4 | 0 | 62 | 78 | 0 | 0·0 | 0 |
| 6 | 1:4 | 98 | 62 | 195 | 80 | 32·0 | 24 |
| 7 | 2:4 | 210 | 62 | 273 | 200 | 64·0 | 48 |

* One unit of N or P in the index represents the concentration of N (as $NO_3$) or P found in half–strength Hoagland's nutrient solution.

& Maronek, 1981; Gruhn, Roncadori & Kormanik, 1987) or increase (Sylvia & Schenck, 1983; Johnson, 1984) sporulation.

Experiments were conducted to explore the hypothesis that nutrient treatments which enhance colonization of roots by VA mycorrhizal fungi should also increase sporulation by those fungi. A variety of nutrient solutions, chosen to yield a wide range of growth and colonization, were applied to bahiagrass (*Paspalum notatum* Flügge) seedlings colonized with one of three VA mycorrhizal fungi.

## MATERIALS AND METHODS

### *Experimental material*

Spores of *Gigaspora margarita* Becker & Hall (INVAM 185)*, *Acaulospora longula* Spain & Schenck (INVAM 316), and *Glomus intraradix* Schenck & Smith (INVAM 208) were isolated from pot culture soil (Arredondo fine sand; loamy siliceous, hyperthermic Grossarenic Paleudult) and used to inoculate seedlings of *Paspalum notatum* Flügge. Twenty to 40 spores were placed on a cellulose filter approximately 5 cm below the surface of soil in a 165 cm$^3$ conical plastic pot ('cone–tainer', Ray Leach 'Cone–tainer' Nursery†, Canby, OR 97013, USA). One *P. notatum* seedling was transplanted into each pot. Plants were grown in a growth chamber under artificial light (60–800 $\mu$mol m$^{-2}$ s$^{-1}$ PAR, 14/10 h day/night) for 8 wk during which only water was applied.

After 8 wk in the growth chamber, seven plants and pot culture soils for each of the VA mycorrhizal fungi studied were sampled for spore populations and percentage root length colonized by VA mycorrhizal fungi (see 'Collection of Data'). Remaining plants were moved to a glasshouse where they were grown under natural photoperiods from 26 April to 14 July. Seven nutrient solutions were applied to plants colonized with each of the VA mycorrhizal fungi (3 × 7 × 7; VA mycorrhizal fungi × nutrient solutions × replications, respectively). Blocks of plants receiving a treatment were randomized in the glasshouse and rearranged several times throughout the experiment. Twenty ml of nutrient solutions were added three times weekly. Solutions ranged from tap water (adjusted to pH 6·8 with 0·25 M $H_2SO_4$) to Hoagland's solution with twice the prescribed concentration of P (Hoagland & Arnon, 1938) (Table 1). Final pH of all solutions ranged from 6·6 to 6·8. The micronutrient solution described in Hoagland & Arnon (1938) was added to each nutrient solution.

### *Collection of data*

At the end of the experiment, tissue from plants colonized by *A. longula* and *G. intraradix* was dried (105 °C), ground to pass a 20-mesh screen, and analysed for N, P, K, and ethanol-soluble sugars. Nitrogen and K were determined in the digestate resulting from a $H_2SO_4$ + selenized granule (Hengar Granules; Hengar Co., Philadelphia, PA, USA) wet digestion on an aluminum block. Nitrogen was determined colorimetrically (Wall & Gehrke, 1975) and K by atomic absorption. Phosphorus was quantified in tissue ashed in a muffle furnace. Ash was solubilized in 1·0 M HCl and P was determined colorimetrically using the ascorbic acid–molybdenum blue method (Murphy & Riley, 1962). Ethanol–soluble sugars were extracted by shaking 50 mg of ground tissue in 30 ml 80% ethanol for 30 min at room temperature. Sugars were quantified using the phenol-sulphuric acid method (Dubois *et al.*, 1956) and expressed as glucose equivalents.

Soilborne spores of *G. margarita* and *A. longula* were isolated by wet sieving (Gerdemann & Nicolson, 1963) and centrifugation (Jenkins, 1964) using 43 and 21 cm$^{-3}$ sections of soil, respectively, from the middle of the pots. Intraradical spores of *G.*

* International Culture Collection of VA Mycorrhizal Fungi (INVAM) isolate number.

† Use of a product name does not constitute an endorsement by the Florida Institute of Food and Agricultural Sciences.

**Table 2.** *Concentrations of nutrients and pH of soil in rhizospheres of* Paspalum notatum *colonized by VA mycorrhizal fungi and fertilized three times per week with one of seven nutrient solutions. Data represent the means of three pooled samples, one each from plants colonized with* Acaulospora longula, Glomus intraradix, *and* Gigaspora margarita*

| Solution number | N:P index | Concentration (mg kg soil$^{-1}$) | | | |
|---|---|---|---|---|---|
| | | $NO_3$ | P | K | pH |
| 1 | 0:0 | 5·5±0·4 | 12·4±1·2 | 4·0±0·8 | 5·8 |
| 2 | 2:0 | 38·6±5·3 | 7.6±0·0 | 79·7±3·0 | 6·4 |
| 3 | 1:2 | 15·4±0·4 | 20·5±1·9 | 30·6±2·4 | 6·9 |
| 4 | 2:2 | 20·6±2·1 | 21·9±2·1 | 73·7±4·5 | 7·1 |
| 5 | 0:4 | 9·1±3·1 | 73·1±2·1 | 41·7±0·5 | 5·8 |
| 6 | 1:4 | 10·1±1·0 | 75·9±3·4 | 31·2±5·0 | 6·7 |
| 7 | 2:4 | 23·5±7·6 | 108·4±1·0 | 76·7±14·6 | 7·3 |

* See Table 1 for composition of nutrient solutions.

*intraradix* were counted after clearing all 0·5 cm root pieces from the borders of the upper and lower thirds of the pot in 10% (w/v) KOH for 2 h. Percentage root length colonized by VA mycorrhizal fungi was determined using the gridline–intersect method (Newman, 1966) after staining roots used for the enumeration of spores of *G. intraradix* and those within the section of soil sampled for soilborne spores of *G. margarita* and *A. longula* (Phillips & Hayman, 1970).

Data were analysed by linear regression and analysis of variance. Characteristics for which significant treatment effects were found were further characterized using Duncan's multiple range test ($\alpha = 0{\cdot}05$). Data for percentage root length colonized were analyzed after arcsin transformation.

**Table 3.** *Physical characteristics of* Paspalum notatum *colonized by* Acaulospora longula *and fertilized three times each week with one of seven nutrient solutions**

| Solution number | N:P index | Dry Weight (g) | | Root:shoot ratio |
|---|---|---|---|---|
| | | Shoot | Root | |
| 1 | 0:0 | 0·73 d | 0·60 c | 0·84 a |
| 2 | 2:0 | 5·91 c | 2·00 b | 0·34 b |
| 3 | 1:2 | 9·10 b | 2·45 a | 0·27 cb |
| 4 | 2:2 | 10·37 a | 2·40 a | 0·23 c |
| 5 | 0:4 | 0·73 d | 0·58 c | 0·79 a |
| 6 | 1:4 | 9·58 ab | 2·35 a | 0·25 c |
| 7 | 2:4 | 10·02 a | 1·94 b | 0·20 c |

* See Table 1 for composition of nutrient solutions. Each number represents the mean of seven observations. Numbers in the same column followed by the same letter are not significantly different ($\alpha = 0{\cdot}05$, Duncan's multiple range test).

## RESULTS

### *Soil and plant characteristics*

Soil nutrient concentrations and pH at the end of the experiment reflected nutrient additions. Addition of solutions containing Ca, N, Mg, and micronutrients increased the pH of the soil (Table 2).

The addition of nutrient solutions with N caused a marked increase in growth of *P. notatum* (Table 3). Data for plants colonized with *A. longula* are representative of the others. The addition of 2 mM $KH_2PO_4$ (solution 5) to plants caused no change in plant mass relative to plants receiving only tap water (solution 1). Nutrient solutions with N and P caused increases in shoot biomass over that of Hoagland's solution without P.

Nitrogen concentrations of roots and shoots reflected nutrient addition (Table 4). Plants receiving solutions 4, 5 and 7 partitioned more N in the shoot relative to the root when colonized by *G. intraradix* than when colonized by *A. longula*. Addition of nutrient solutions without P or those with the lowest level of P (solutions 2–4) resulted in substantial dilution of tissue P (Table 4). Plants colonized with *G. intraradix* had greater P concentrations than those with *A. longula* when 2 mM $KH_2PO_4$ (solution 5) was applied. Nitrogen:phosphorus ratios in plant tissue varied widely depending upon the relative availabilities of N and P (Table 5).

Potassium concentrations in plant tissue were affected little by the species of VA mycorrhizal fungus (Table 4). Potassium was partitioned more toward the shoot for plants in all treatments except $KH_2PO_4$ (solution 5).

Concentrations of soluble sugars in roots were significantly lower in plants receiving Hoagland's solution without P (solution 2) than for other solutions (Table 6). Solutions with additional P (solutions 3, 6 and 7) tended to produce the greatest concentration of soluble sugars in roots of *P. notatum*.

**Table 4.** *Concentrations of nitrogen, phosphorus, and potassium in roots and shoots of* Paspalum notatum. *Roots were colonized with* Acaulospora longula *or* Glomus intraradix *and fertilized three times each week with one of seven nutrient solutions**

| Solution number | Nutrient concentration (percentage of dry weight) | | | | | |
|---|---|---|---|---|---|---|
| | N | | P | | K | |
| | Shoot | Root | Shoot | Root | Shoot | Root |
| | *Acaulospora longula* | | | | | |
| 1 | 1·73 b | 0·61 c | 0·17 a | 0·06 cd | 1·25 d | 0·31 e |
| 2 | 2·55 a | 2·72 a | 0·04 d | 0·03 e | 1·82 b | 1·09 b |
| 3 | 2·10 ab | 1·08 c | 0·08 c | 0·05 d | 1·14 d | 0·58 d |
| 4 | 2·38 a | 2·34 b | 0·06 c | 0·05 d | 1·62 c | 1·27 a |
| 5 | 0·69 c | 0·68 d | 0·19 a | 0·14 a | 1·54 c | 1·23 a |
| 6 | 2·18 ab | 1·28 c | 0·13 b | 0·06 bc | 1·60 c | 0·76 c |
| 7 | 2·15 ab | 2·75 a | 0·12 b | 0·07 b | 2·05 a | 1·16 ab |
| | *Glomus intraradix* | | | | | |
| 1 | 1·03 e | 0·67 e | 0·18 b | 0·08 b | 0·93 e | 0·03 f |
| 2 | 1·99 d | 2·24 a | 0·04 e | 0·03 d | 1.81 bc | 1.03 c |
| 3 | 2·28 bc | 0·95 d | 0·09 d | 0·06 c | 1·28 d | 0·61 e |
| 4 | 2·44 b | 1·69 b | 0·07 d | 0·06 c | 1·98 b | 1·43 a |
| 5 | 1·23 e | 0·67 e | 0·31 a | 0·20 a | 1·70 c | 1·28 b |
| 6 | 2·12 cd | 0·88 d | 0·14 c | 0·07 bc | 1·76 c | 0.92 d |
| 7 | 3·12 a | 1·53 c | 0·13 c | 0·07 bc | 2·23 a | 1.34 ab |

* See Table 1 for composition of nutrient solutions. Each number represents the mean of seven observations. Numbers within a column for a VA mycorrhizal fungus species, followed by the same letter, are not significantly different ($\alpha = 0{\cdot}05$, Duncan's multiple range test).

**Table 5.** *Nitrogen : phosphorus ratios of* Paspalum notatum *colonized by* Acaulospora longula *or* Glomus intraradix *and fertilized three times each week with one of seven nutrient solutions**

| Solution number | *Acaulospora longula* | | *Glomus intraradix* | |
|---|---|---|---|---|
| | Shoot | Root | Shoot | Root |
| 1 | 10·2 de | 11·2 e | 5.8 e | 8·5 e |
| 2 | 66·9 a | 87·3 a | 49·4 a | 69·5 a |
| 3 | 27·3 c | 21·2 d | 25·1 c | 16·7 d |
| 4 | 38·8 b | 51·3 b | 33·8 b | 29·1 b |
| 5 | 3·8 e | 5·0 e | 4·0 e | 3·4 f |
| 6 | 16·6 d | 20·3 d | 15·3 d | 12·9 d |
| 7 | 17·4 d | 38·1 c | 25·0 c | 21·4 c |

* See Table 1 for composition of nutrient solutions. Statistics as in Table 3.

**Table 6.** *Concentrations of ethanol–soluble sugar in roots of* Paspalum notatum *colonized by* Acaulospora longula *or* Glomus intraradix *and fertilized three times each week with one of seven nutrient solutions.**

| Solution number | Soluble sugars ($\mu$g mg root$^{-1}$) | |
|---|---|---|
| | *A. longula* | *G. intraradix* |
| 1 | 93·7 bc | 109·4 a |
| 2 | 58·8 d | 68·1 b |
| 3 | 114·9 ab | 104·0 a |
| 4 | 86·7 c | 104·1 a |
| 5 | 95·5 bc | 98·1 a |
| 6 | 102·9 abc | 109.8 a |
| 7 | 122·1 a | 111·0 a |

* See Table 1 for composition of nutrient solutions. Sugars are expressed as glucose equivalents. Statistics as in Table 3.

### *VA mycorrhizal fungus sporulation and colonization of root systems*

The addition of Hoagland's nutrient solution without P (solution 2) produced the greatest density of soilborne spores of *A. longula* and *G. margarita* (Fig. 1 *a*, *b*). *Glomus intraradix* produced more spores per root weight when tap water or $KH_2PO_4$ (solutions 1 and 5) were applied (Fig. 1 *c*). Hoagland's solutions without P, however, stimulated the production of the same amount of spores of *G. intraradix* per root system as the $KH_2PO_4$ treatment ($22550 \pm 4290$ *vs.* $22450 \pm 2440$; mean $\pm$ SEM for solutions 2 and 5, respectively) and significantly more than the water only treatment ($12850 \pm 2240$). The addition of Hoagland's solution without P (solution 2) caused a significant increase in percentage root length colonized for all species of VA mycorrhizal fungi studied (Fig. 2 *a–c*). Addition of 2 mM $KH_2PO_4$ did not prohibit colonization of roots by these fungi.

Spore populations in the soil were correlated with

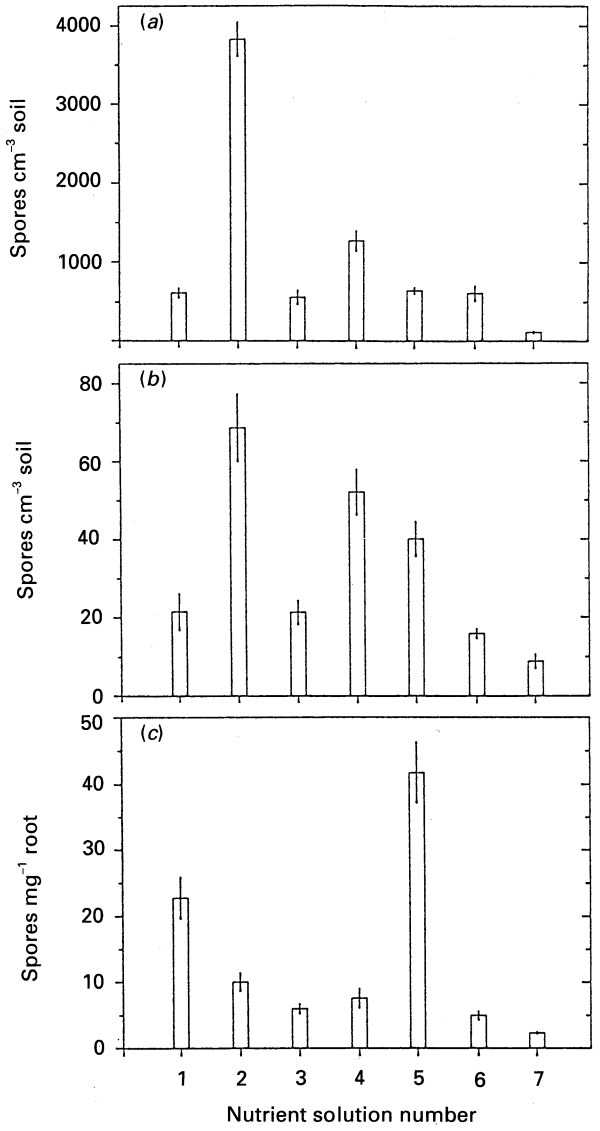


**Figure 1.** Spore densities of (*a*) *Acaulospora longula*, (*b*) *Gigaspora margarita*, and (*c*) *Glomus intraradix* produced in mycorrhizal association with *Paspalum notatum* receiving various nutrient solutions. See Table 1 for composition of nutrient solutions. Bars represent the means of seven observations ± SEM.

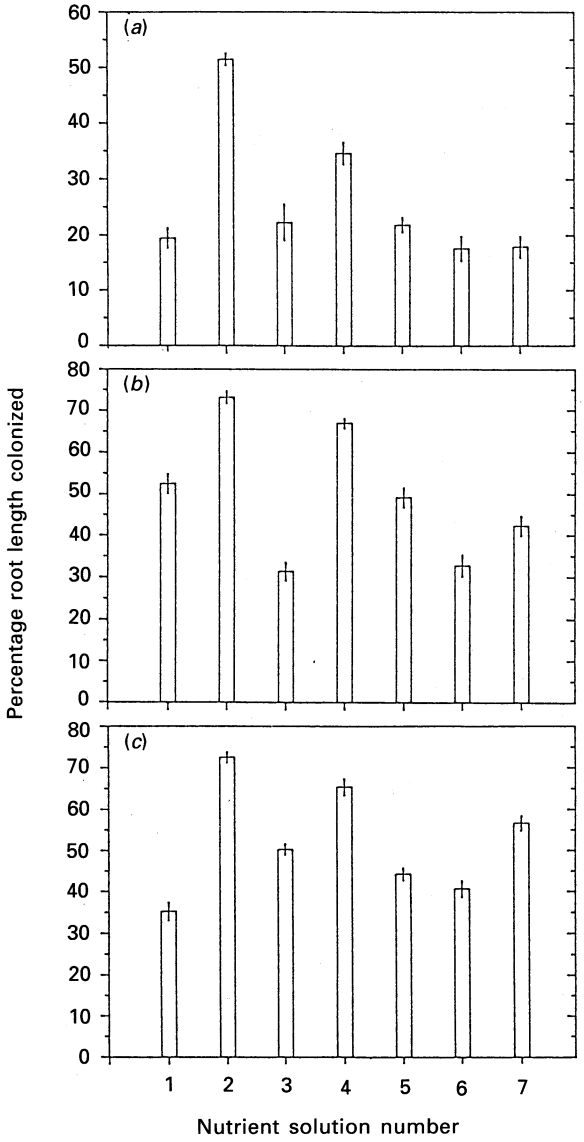


**Figure 2.** Percentage root length of *Paspalum notatum* colonized by (*a*) *Acaulospora longula*, (*b*) *Gigaspora margarita*, and (*c*) *Glomus intraradix*. See Table 1 for composition of nutrient solutions. Bars represent the means of seven observations ± SEM.

different factors from spore populations within roots. Spore density in the soil was significantly and positively correlated to percentage root length colonized for *G. margarita* and *A. longula* ($r^2 = 0{\cdot}599$ and $0{\cdot}824$, $P < 0{\cdot}0001$, respectively) but not for *G. intraradix* spores per mg root ($r^2 = 0{\cdot}102$). Spore production by *G. intraradix* per weight of root was positively correlated to N content of the shoot and root ($r^2 = 0{\cdot}517$, $0{\cdot}591$, and $0{\cdot}654$, $P < 0{\cdot}0001$, respectively). Sporulation of *A. longula* did not correlate well with N, P, or K concentrations in the plant. Inclusion of N, P, and K in regression equations predicting spore densities produced correlation coefficients of $0{\cdot}366$ and $0{\cdot}327$ for shoot and root concentrations, respectively. Sporulation of *A. longula* was correlated to N:P ratios of shoot and root ($r^2 = 0{\cdot}668$ and $0{\cdot}674$, $P < 0{\cdot}0001$, respectively), but this correlation was not significant for *G. intraradix* ($r^2 = 0{\cdot}124$ and $0{\cdot}276$ for shoot and root N:P ratios, respectively). *Glomus intraradix* produced the most spores per root weight when N:P ratios were less than 10 (Table 5 and Fig. 1*c*). Sporulation of *A. longula* was significantly correlated with root soluble sugar concentration ($r^2 = 0{\cdot}500$). The reverse occurred with *G. intraradix*. Spores per weight of root were correlated with sugar concentration in roots of *P. notatum* ($r^2 = 0{\cdot}006$).

Percentage root length colonized was correlated more with P concentration of shoots than of roots for *A. longula* [$r^2 = 0{\cdot}410$ ($P < 0{\cdot}0001$) *vs.* $0{\cdot}177$ ($P < 0{\cdot}003$), respectively] and *G. intraradix* [$r^2 = 0{\cdot}396$ ($P < 0{\cdot}0001$) *vs.* $0{\cdot}182$ ($P < 0{\cdot}0023$), respectively]. Colo-

nization of roots by *G. intraradix* was positively correlated with N concentration of roots ($r^2 = 0{\cdot}792$, $P < 0{\cdot}0001$). Colonization of roots by *A. longula* was correlated with concentration of sugars in roots ($r^2 = 0{\cdot}479$, $P < 0{\cdot}0001$). Colonization by *G. intraradix* was correlated with neither root soluble sugar content nor concentration.

## DISCUSSION

The responses of the VA mycorrhizal fungi studied here suggest these fungi may divide into two groups with respect to factors affecting sporulation. Sporulation of *G. intraradix* within the roots of *P. notatum* was not correlated with degree of colonization of the root system, whereas soilborne populations of *A. longula* and *G. margarita* were. Nor was sporulation of *G. intraradix* correlated with tissue N:P ratios or root soluble sugar concentrations. Soilborne populations of *A. longula* were correlated with these characteristics. Sporulation of *G. intraradix* was correlated with P concentrations in the root and shoot. Sporulation of *A. longula* was not correlated with these factors. *Glomus intraradix* was shown to be 'P–tolerant' (Sylvia & Schenck, 1983). The correlations listed above may distinguish VA mycorrhizal fungi as P tolerant or intolerant with respect to sporulation. Phosphorus–tolerant species can be characterized as exhibiting sporulation that is independent of degree of colonization, tissue N:P ratios, and root soluble sugar concentrations.

Nutrient addition affected percentage root length colonized similarly for all species. Water, and those solutions in which P concentrations were above those in a balanced Hoagland's solution (solutions 1, 3, 5–7), produced less colonization than when N and P were in balance or solution without P was added (solutions 4 and 2, respectively). We found no evidence for $NO_3$-induced depression of percentage colonization due to increased root growth rate (Seo, Anderson & Liberta, 1988).

The different strategies employed by *A. longula* and *G. margarita* as opposed to *G. intraradix* may reflect different pathways for transport of carbon to sporulating hyphae. All of these fungi are obligate symbionts and require fixed carbon from the host plant. Spores are rich in lipid (Beilby & Kidby, 1980) and sporulation would require a large supply of carbon. *Acaulospora longula* and *G. margarita* produce spores in the soil, terminally and singly, on hyphae which must lead back to the source of carbon, and arbuscule. Sporulation of these fungi, therefore, would be expected to increase with increasing colonization of the root system, as seen here. *Glomus intraradix* produces spores within the host root. The path of carbon flow from arbuscule to developing spore is much shorter and less colonization may be necessary. Spores of *G. intraradix* also tended to be produced in clusters, further minimizing the need for widespread colonization. This does not explain why *G. intraradix* did not produce still more spores per root weight or length when the roots were heavily colonized. The 'P–tolerance' of *G. intraradix* may be primarily a response to increase sporulation under conditions of stress such as high P or low nutrient availability.

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